IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: LINHART, et al

Serial No.: 10/026,914

Attorney Docket No.: 966927.00006

Filing Date: December 27, 2001

Examiner: Hines, Jana A

Art Unit: 1645

Allergy Vaccines Containing Hybrid Polypeptides

APPEAL BRIEF

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Dear Sir:

The above-identified patent application comes before the United States Patent and Trademark Office Board of Appeals and Interferences from the Final Rejection of Claims 42-43, 45-47 by the Examiner in an Official Action mailed June 20, 2007. Pursuant to the Notice of Appeal and Pre-Appeal Brief Request for Review filed August 15, 2007, set forth below is the Appellant's Brief. A Check in the amount of $255.00 is herewith submitted for payment of the fee under 37 C.F.R. §41.20(b)(2).

The Commissioner is hereby authorized to charge any fees which may be required during the entire pendency of the appeal, or credit any overpayment, to Deposit Account 50-4336.

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I. Real Party in Interest:

The real party in interest in the above-captioned application is BIOMAY PRODUKTIONS-und Handels-Aktlengessellschaft Corp. ("Appellant"), a corporation of the Country of Austria, and having a place of business at Lazarettgasse 19, 1090 Vienna, Austria. The application has been assigned of record to SHAN-Beteiligungsgesellschaft mbH by the inventors: Birgit Linhart; Dietrich Kraft; and Rudolf Valenta; said assignee of record having merged with the Real Party in Interest by virtue of a merger agreement executed December 02, 2002.

II. Related Appeals and Interferences:

There are no appeals or interferences known to Appellant or Appellant's legal representative which will directly affect or be directly affected by or have a bearing on the Board's decision in this present appeal.

III. Status of Claims:

Claims 7, 9, 22-25 and 36-47 are pending. Claims 7, 9, 22-25, 36-41, 44 were withdrawn and claims 1-6, 8, 10-21, 26-35, and 48-51 were cancelled. Claims 42-43 were finally rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement, in an Office Action mailed June 20, 2007. Claims 42-43 and 45-47 were finally rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 45-47 were finally rejected under 35 U.S.C. §103(a) as being unpatentable over Ball et al., (WO 95/34578) in view of Vrtala et al., (1996. J. Allergy Clin. Immun. Vol. 97(3); 781-787). Claims 42-43 and 45-47 are the subject of the present appeal.

IV. Status of Amendments:

No amendments have been filed subsequent to the Final Rejection. All amendments have been entered and are reflected in the claims appendix.

V. Summary of Claimed Subject Matter:

As recited in independent claim 42, the present invention is directed to a method of preparing fusion polypeptides consisting of timothy grass pollen allergens for use as immunotherapeutic agents; said method comprising the steps of: (a) providing a polynucleotide sequence encoding the fusion polypeptide, See Figure 2. See also Example 2, page 11; (b) introducing said polynucleotide sequence into a host cell, See Example 3, Page 13; (c) culturing the host cell obtained in b) under conditions such that the fusion polypeptide is expressed, See Example 3, page 13, paragraph 3; and (d) recovering the expressed fusion polypeptide from the cultured host cell, See Example 3, page 13, paragraph 4; (e) testing the fusion polypeptide as candidate immunotherapeutic agents by administering said polypeptide to a test animal and selecting as immunotherapeutic agents those fusion polypeptides that induce IgE-blocking antibodies and induce stronger immune responses compared with the individual components or fragments thereof, See Example 5, page 15. See also Example 6, page 16. Claim 43 depend from claim 42 and further define the use of PCR technology to obtain the polynucleotide sequence encoding the timothy grass pollen polypeptides.

Independent claim 45 recites a pharmaceutical composition comprising one or more fusion allergens of timothy grass pollen allergens for use as immunotherapeutic agents, wherein said agents consists of fusion allergens of timothy grass pollen allergens which have been identified by a method comprising the steps of: (a) providing fusion allergens of naturally occurring timothy grass pollen allergens, See Figure 2. See also Example 2, page 11; (b) challenging an immunological model with said fusion allergens See Example 5, page 15. See also Example 6, page 16; (c) selecting as candidate immunotherapeutic agents, those fusion allergens which induce IgE-blocking antibodies and have reduced allergenic activity compared

with the respective allergens which comprise the fusion allergen See Example 5, page 15. See also Example 6, page 16. Claim 47 depends from claim 45 and is directed to a pharmaceutical composition comprising fusion proteins selected from the group consisting of timothy grass pollen allergens rPhl p 1, rPhl p 2, rPhl p 5, and rPhl p 6.

Claim 46 is directed to a hybrid allergen for treatment of IgE-mediated hypersensitivity, wherein said hybrid allergen is a fusion protein consisting of two or more timothy grass pollen allergens.

Prior to the claimed invention, allergen-specific immunotherapy was based on systemic administration of crude, ill-defined, non-standardizeable allergens extracts to induce allergen-specific unresponsiveness a great risk for anaphylactic side effects and without regards to the specific sensitization profile of a given patient. The claimed invention, among other things, has overcome the problems of the prior art related to the use of non-standardizeable crude allergen extracts by using allergens of well-defined composition. Furthermore, the inventors have demonstrated for the first time that hybrid fusion polypeptides derived by assembling the epitopes of immunologically distinct allergens can be used for diagnosis and treatment of Type I allergic disorders. See Specification, p.2, paragraph 2. Candidate hybrid polypeptides can comprise at least one complete allergen protein; or two different complete allergen proteins coming from same or different sources. See Specification, p2, paragraph 6. The claims under consideration are, however, limited to hybrid allergens derived from timothy grass pollen allergens and encompass a method of preparing hybrid fusion timothy grass pollen polypeptides and pharmaceutical compositions directed thereto for use in allergy immunotherapy.

VI. Grounds of Rejection to be Reviewed:

Issue 1

Whether claims 42 - 43 comply with the written description requirement under 35 U.S.C. §112, first paragraph.

Issue 2

Whether claims 42-43 and 45-47 are indefinite under 35 U.S.C. §112, second paragraph as allegedly failing to particularly point out and distinctly claim the subject matter with applicant regards as the invention.

Issue 3

Whether claims 45-47 are unpatentable under 35 U.S.C. §103(a) over Ball et al., (WO 95/34578) in view of Vrtala et al., (1996. J. Allergy Clin. Immun. Vol. 97(3); 781 -787).

VII. Grouping of Claims:

There are three groups of claims, which stand or fall separately. Group 1 consists of independent claim 42 and dependent claim 43. Group 2 consists of independent claim 45 and dependent claim 47. Group 3 consists of independent claim 46.

VIII. Argument:

Issue 1

Whether claims 42 - 43 comply with the written description requirement under 35 U.S.C. §112, first paragraph.

The Examiner recognized that claims 42-43 are drawn to a **method** of preparing fusion polypeptides consisting of timothy grass pollen allergens for use as immunotherapeutic agents. See June 06, 2005, Final Office Action, page 2. Incongruously, however, the Examiner asserts that "[n]o information, beyond the characterization of a polypeptide having the ability to encode a fusion polypeptide have been provided, which would indicate that applicants did not have possession of the claimed genus of any polypeptide sequences." *Id.* 3, paragraph 2. The Examiner further asserts that the specification does not contain the disclosure of the structure of **all** polynucleotide sequences that encode a fusion polypeptide, which is within the scope of the claimed genus. The Examiner further asserts that there is no disclosure of the **exact make-up** of the fusion polypeptide nor does the specification provide for the **structure of the polynucleotide,** nor the structural characterization of the **complete sequence.** *Id.* at 4.

Further incongruously, the Examiner asserts that the **method step** regarding selecting as immunotherapeutic agents those fusion polypeptides that induce IgE-blocking antibodies and induce stronger immune responses compared with the individual components or fragments thereof is a mere functional characterization the polynucleotide and "does not describe the polynucleotide itself." *Id.* at 5.

Further incongruously, the Examiner cites MPEP 2163 as requiring in a method claim, that the disclosure of a biomolecule by functional characteristics, without any disclosed correlation between function and structure is not sufficient characteristics for written description purposes. *Id.* at 5.

Further incongruously, the Examiner asserts that the specification fails to teach what the

(sic) critical nucleic acids can or cannot be modified and still achieve a functional fusion polypeptide and thus fails to enable the skilled artisan to envision the **detailed chemical structure** of the claimed structure **of the claimed polynucleotide**. Id. at 6.

Further incongruously, the Examiner asserts that method steps comprises in relevant part, "providing "a" polynucleotide encoding the fusion polypeptide," and that "this reads on a single nucleotide as having the ability to encode the fusion polypeptide," and that the specification did not disclose a single nucleotide having the ability to encode the fusion polypeptide consisting of timothy grass pollen allergens. Id. at 7.

The Examiner further asserts that although claim 42 refers to individual components or fragments thereof, that there is no description of the individual components or fragments thereof. *Id.* at 7.

The Examiner summed up this ground for rejection by stating that "the nucleic acid itself is required." *Id.* at 7.

Responding to arguments presented in the March 27, 2007, Response and Amendments, in which Applicants, had amended the claims to recite "a polynucleotide sequence" in order to obviate the Examiner's incongruous remarks that "a polynucleotide" reads on "a single nucleotide," the Examiner stated that the specification had not explicitly defined a "polynucleotide" and thus "a polynucleotide sequence" had no lower limit on the size of the polynucleotide. *Id.* at. 9 and 10.

Responding to arguments presented in the March 27, 2007, Response and Amendments in which Applicants directed the Examiner to Figure 2 and Example 2 as showing the **construction** of recombinant hybrid allergens, the Examiner, inter alia, asserted that the specification does not contain a structural characterization of the complete sequence. *Id.* at 11. Applicant traverses this rejection because the method of preparing fusion polypeptides of timothy grass pollen allergen is described in the specification in such a way as to reasonably convey to one of ordinary skill in

the art at the time the application was filed that the Applicant had possession of the claimed invention as required by 35 U.S.C. §112, first paragraph.

35 U.S.C. §112, first paragraph sets forth in part:

> the specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The MPEP summarizes the Federal Circuit's characterization of the written description requirement by stating that, "[a]n applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention." MPEP §2163 *citing Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997). Further, the MPEP states that "[p]ossession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was 'ready for patenting' such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention." MPEP §2163 *citing Pfaff v. Wells Electronics, Inc.*, 525 U.S. 55, 68 (1998); *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1568, (Fed. Cir. 1997), *cert. denied*, 523 U.S. 1089 (1998); and *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206 (Fed. Cir. 1991).

a. The Claimed Invention is a Methodology and it is Clearly Erroneous to Examine it as if it were a Product Claim

The Examiner correctly recognizes that claims 42-43 are drawn to a **method** of preparing fusion polypeptides consisting of timothy grass pollen allergens for use as immunotherapeutic agents, but quite incongruously requires that the inventors must disclose the primary structure, down to exact nucleotide or amino acid sequence of representative, if not, all polynucleotides or polypeptides that can be used to practice the invention. Applicants believe that Application of 35 U.S.C. 112, first paragraph, must start with proper construction of the claimed subject matter and inquiry as to whether or not the claimed subject matter is described to patentably enabling details should be based on what is claimed.

A method claim by definition discloses how to accomplish a claimed objective and the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same.

Independent claim 42, is directed to a method of preparing fusion polypeptides consisting of timothy grass pollen allergens for use as immunotherapeutic agents; said method comprising the steps of: (a) providing a polynucleotide sequence encoding the fusion polypeptide, See Figure 2. See also Example 2, page 11; (b) introducing said polynucleotide sequence into a host cell, See Example 3, Page 13; (c) culturing the host cell obtained in b) under conditions such that the fusion polypeptide is expressed, See Example 3, page 13, paragraph 3; and (d) recovering the expressed fusion polypeptide from the cultured host cell, See Example 3, page 13, paragraph 4; (e) testing the fusion polypeptide as candidate immunotherapeutic agents by administering said polypeptide to a test animal and selecting as immunotherapeutic agents those fusion polypeptides that induce IgE-blocking antibodies and induce stronger immune responses compared with the individual components or fragments thereof, See Example 5, page 15. See also Example 6, page 16.

The Examiner is not contending that the Applicants did not have possession of this five-step process; nor is the Examiner contending that these steps do not accomplish the claimed objective; instead the Examiner is contending that the molecularity of the chemical species practiceable with the invention must be disclosed as well. The Examiner keeps insisting that the Applicants must show that they "had possession of the claimed genus of any polypeptide sequences," or that "the specification must contain the structure of all polynucleotide sequences that encode a fusion polypeptide," or that the "exact make-up" of the fusion polypeptide be disclosed; whereas all the Applicants are claiming is a method of preparing fusion polypeptides consisting of timothy grass pollen allergens, said method steps adequately and enablingly described in the specification.

The specification is replete with teachings of how to accomplish the claimed methodology exemplified by the major timothy grass pollen allergens rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 6, the disclosure of which constitutes an adequate representation of the genus of timothy grass pollen allergens. Applicants believe that the specification adequately describes a method of preparing a hybrid polypeptide using hybrid polynucleotide sequences encoding said hybrid polypeptide. Reference is made to Figure 2 wherein the method of producing a hybrid polynucleotide of Phl p 5 and Phl p 1 is reduced to drawing. On page 11, Example 2, Applicants describe in detail how to construct recombinant hybrid allergens. On page 13, Example 3, Applicants describe how to obtain a recombinant hybrid polypeptide using the recombinant hybrid polynucleotide sequences of Example 2.

The Patent and Trademark Office provides examiners with a set of guidelines to follow when examining patent applications for compliance with the written description requirement called the "Revised Interim Written Description Guidelines Training Material" ("Guidelines"). *See* 66 Fed. Reg. 1099, 1099-1111 (January 5, 2001). The Guidelines state that "[t]here is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed." 66 Fed. Reg. 1099, 1105. Further, the Guidelines state that the, "[w]ritten description for a claimed genus may be satisfied . . . by disclosure of relevant,

identifying characteristics, i.e. structure or other physical and/or chemical properties . . . or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus." *Id.* at 1106. The Guidelines also provide that, "[t]he absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. [§]112, [paragraph] 1, for lack of adequate written description." 66 Fed. Reg. 1099, 1105. Following the teaching of the Guidelines, the written description requirement is satisfied by either identifying the features of the invention, such as its physical and/or chemical properties or by using well-known terms of art as the Applicants have done in this case.

b. The Claimed Methodology is Heuristic and the Examiner Failed to Appreciate the Fundamental Nature of the Claims

By heuristic, Applicants believe that they had in their possession, as at the time of filing this Application, a method of discovering fusion polypeptides consisting of timothy grass pollen allergens that can be used as immunotherapeutic agents against Type I allergies caused by timothy grass pollens.

By definition in this context, a **heuristic** is a technique designed to solve a problem that ignores whether the solution can be proven to be correct, but which usually produces a good solution or solves a simpler problem that contains or intersects with the solution of the more complex problem. A method of making an invention is in and of itself, useful, inventive, and patentable and the Examiner has failed to recognize this fundamental character of the invention.

Prior to this invention, the conventional method of desensitization of allergy patients is by the use of systemic administration of crude, nonstandardizeable allergen extracts without regards to their exact chemical composition. On the other hand, great amounts of scientific endeavors have also been put forth in the characterization of known allergens, their epitopic

mapping; their primary, secondary, and tertiary structure characterization; as well as experimentally onerous substitution, addition, and deletion studies aimed at potentiating natural allergens or derivatives thereof for use as immunotherapeutic agents. It does clearly appear as if the Examiner, informed by the degree of onerousity of those experimentally intensive routes, imposes a standard of 35 U.S.C. 112, first paragraph, that is inappropriate for the claimed invention under review.

Not only have scientists wrestled with the experimentally onerous characterization and potentiation of known allergens for use as immunotherapeutic agents, there is also a need for cross-sensitization of patients across a broad class of allergens by using the least amount of standardizeable allergen derivatives. The thinking prior to the instant invention is that this can be accomplished by systemic administration of a cocktail of standardized allergen derivative.

The invention under consideration is premised on the principle that rather than making, purifying, and standardizing a cocktail of allergen derivatives, that hybrid polypeptides or fusion allergens consisting of those individual allergens or their derivatives can and surprisingly could be used as immunotherapeutic agents. Moreover, these hybrid polypeptides do not necessarily have to come from one plant or animal species in order to impart cross-sensitization across a broad range of allergens. The inventors of the instant invention were first to discover that hybrid polypeptides or fusion polypeptides can be useful immunotherapeutic agents.

The second leg on which this heuristic methodology stands is that rather than go the experimentally undue and onerous route of molecularly characterizing and epitopically mapping, substituting, adding, deleting, and derivatizing these hybrid allergens or fusion polypeptides, that one could simply follow the far less experimentally involved step (e) of the claimed methodology which is testing the fusion polypeptide as candidate immunotherapeutic agents by administering said polypeptide to a test animal and selecting as immunotherapeutic agents those fusion polypeptides that induce IgE-blocking antibodies and induce stronger immune responses compared with the individual components or fragments thereof, See Example 5, page 15. See also Example 6, page 16.

In other words, the inventors now have in their possession, the equivalent of an immunological litmus test used as a heuristic; a technique designed to solve the problem of immunotherapeutic agents that ignores whether the structure of the resulting agent can be proven in the contemporary sense to have its epitopes favorably positioned as such, but which technique produces a good solution or solves a simpler problem that contains or intersects with the solution of the more complex problem. Granted, step (e) involves some amount of experimentation but it is a mere routine, a mere litmus test; not one that can be remotely characterized as undue given the routes which scientists have heretofore followed and it is this failure to appreciate that this invention is in and of itself, a rapid method of discovering immunotherapeutic agents consisting of hybrid or fusion polypeptides, that informs the Examiner's manifestly erroneous application of 35 U.S.C. 112, first paragraph.

c. The Claimed Methodology is Inherently Self-Validating and the Methodology does not Stand or Fail on the Exact Molecularity of the Hybrid Polypeptides or Derivatives Thereof

If the Applicants were claiming a product, then adequate inquiry must be had as to whether they had the claimed product in their possession as at the time of filing of the Application. On the other hand, if the Applicants are claiming a method, then the inquiry as to whether they had possession of the method as at the filing of the Application need not exceed the metes and bounds of the claimed method; especially where as here, those metes and bounds have been clearly and concisely delineated.

Applicants are not claiming to be the first to make hybrid polypeptides nor should the method of making such hybrids, which was routinely done and well known to one of skill in the art be used to burden the Application. Applicants are also not claiming to be the first to derivatize proteins by substitution, deletion, or insertion of whole fragments, nor should the method of making such derivatives, which was routinely done and well known to one of skill in the art be used to burden the Application. The obligation to concisely state what they have

invented, invites the obligation to exclude materials which are quite trite while giving notice to one of skill in the art as to exactly what they had possession of.

In regards, therefore, to the heuristic nature of the claimed invention, it is clear and manifest error to insist that guidance as to molecularity of species amenable to the methodology, other than the restrictions appearing on the face of the invention, be provided, where as here, the invention itself is self-validating. In other words, **however derived**, and whatever the molecularity, be it a two amino-acid sequence or five-hundred amino acid sequence, a hybrid polypeptide of timothy grass pollen allergens can now, by virtue of this invention, be for the first time, **routinely tested** as candidate immunotherapeutic agents by administering said polypeptide to a test animal and selecting as immunotherapeutic agents those fusion polypeptides that induce IgE-blocking antibodies and induce stronger immune responses compared with the individual components or fragments thereof, See Example 5, page 15. See also Example 6, page 16.

By teaching for the first time that hybrid allergens can be useful immunotherapeutic agents and by arming investigators with a litmus test for validating the immunotherapeutic utility of hybrid allergens or hybrid derivative allergens, it is manifest error to require *a priori*, that the molecularity of the chemical species amenable to the claimed methodology be the appropriate subject of the first inquiry where as here the steps in the process inherently validate and thus obviate the need for said first inquiry by means of the equivalence of a mere immunological litmus test.

On the basis of the foregoing, particularly the Examiner's failure to properly characterize the invention and the clearly erroneous misapplication of 35 USC 112, first paragraph, this ground for rejection should be removed.

Issue 2

Whether claims 42-43 and 45-47 are indefinite under 35 U.S.C. §112, second paragraph as allegedly failing to particularly point out and distinctly claim the subject matter with applicant regards as the invention.

The Examiner rejected claims 42-43 and 45-47 as indefinite under 35 U.S.C. §112, second paragraph as allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner asserts that the limitation in claim 42 of "the individual components or fragments thereof" lacks sufficient antecedent basis and that it is unclear what the individual components are nor is it clear what the fragments thereof is referring to.

Applicants believe that the foregoing, particularly with respect to claim 42 being heuristic as it were and inherently self validating, that fragments of allergenic proteins can be the proper material for claim 42's methodology. Applicants have also pointed out that a fusion polypeptide is inherently and necessarily an end-to-end fusion of individual polypeptides and thus "the individual components" necessarily mean the polypeptides derived from naturally occurring allergens which have been fused together. Applicants do not believe that one of ordinary skill in the art will have any ambiguity as to whether a fusion polypeptide inherently contains individual components in an end-to-end fashion.

Further, the Examiner asserts that there is insufficient antecedent basis for "the timothy grass pollen polypeptide" in claim 43. Applicants believe, however, that whereas claim 43 depends from claim 42, and whereas claim 42 recites in pertinent part, "a method of preparing fusion polypeptides consisting of timothy grass pollen allergens ..." the antecedent basis is

sufficient to support the claimed limitation in claim 43 and this ground for rejection should be dropped.

Further, the Examiner asserts that the phrase in claim 45 referring to "the respective allergens which comprise the fusion allergen" is unclear. The Examiner further asserts that "it is unclear how the respective allergens which naturally has only one allergen will comprise a fusion allergen" June 20, 2007, Office Action, page 13. With all due respect, Applicants are at a complete loss at to whether the Examiner did in fact, advert her mind to the teachings of the invention, particularly to Figure 2 of the invention which shows the construction of a Phl p5 and Phl p1 fusion of respective timothy grass allergens, where respective in this case refers to Phl p 5 or Phl p1 each standing alone. If a fusion protein as is commonly understood in the art is an end-to-end joining of two proteins; Applicants do not share the Examiner's sense of ambiguity with respect to how a fusion allergen is respectively comprised of other allergens.

Applicants had argued that inherent components of elements recited have antecedent basis in the recitation of the components themselves. MPEP § 2173.05(e). The MPEP provides an example: "the limitation 'the outer surface of said sphere' would not require an antecedent recitation that the sphere has an outer surface. Again, in a clearly erroneous application of the law, the Examiner asserts that "the doctrine of inherency refers (sic) the express, implicit, and inherent disclosures of a prior art reference may be relied upon in the rejection of claims under 35 U.S.C. 102 or 103." The "doctrine of inherency" relied upon by the Applicant is within 35 U.S.C. 112, Second paragraph jurisprudence and not under 35 U.S.C. 102 or 103. Again, this basis for rejection should be dropped.

Issue 3

Whether claims 45-47 are unpatentable under 35 U.S.C. §103(a) over Ball et al., (WO 95/34578) in view of Vrtala et al., (1996. J. Allergy Clin. Immun. Vol. 97(3); 781 -787).

Claims 45-47 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Ball et al. (WO 95/34578) in view of Vrtala et al. (1996. J. Allergy Clin. Immun., Vol. 97(3): 781 - 787). According to the Examiner, Ball et al. teach that the major grass pollen Phl pl can be part of a hybrid or fusion polypeptide but does not specifically recite using another plant allergenic protein within the hybrid polypeptide. As explicitly admitted by the Examiner, Ball et al. "do not teach fusion proteins consisting of two or more timothy grass pollen allergens." June 20, 2007, Final Office Action, p 16.

However, to cure the deficiency in Ball, the Examiner asserts that Vrtala et al., teach that DNA coding for three major timothy grass pollen allergens representing group I (Phl pl), group II (Phl p2) and group V(Phl p5) was known. Therefore, concludes the Examiner, "it would have been prima facie obvious at the time of applicants' invention to apply Vrtala et al's recombinant Pl p1, Phl p2 and Phl p5 to Ball et al's pharmaceutical composition or hybrid allergen in order to enhance antigenicity." *Id.* at 17. Applicants will now traverse.

The Examiner appears to be driven by impermissible hindsight to misconstrue the teachings of the prior art in order to manufacture a clearly erroneous basis for obviousness.

35 U.S.C. §103(a) sets forth in part:

> [a] patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject mater as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said matter pertains.

Section 103 forbids issuance of a patent when "the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art, and (4) where in evidence, so-called secondary considerations. Graham v. John Deere Co., 383 U.S 1, 17-18, 148 USPQ 459, 467 (1966). See also KSR, 127 S.Ct. at 1734, 82 USPQ2d at 1391 ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.") The Court in Graham further noted that evidence of secondary considerations, such as commercial success, long felt but unsolved needs, failure of others, etc., "might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented." 383 U.S. at 18, 148 USPQ at 467.

In KSR, the Supreme Court emphasized "the need for caution in granting a patent based on the combination of elements found in the prior art," *Id.* at 1739, 82 USPQ2d at 1395, and discussed circumstances in which a patent might be determined to be obvious without an explicit application of the teaching, suggestion, motivation test. In particular, the Supreme Court emphasized that "the principles laid down in Graham reaffirmed the 'functional approach' of Hotchkiss, 11 How. 248." KSR, 127 S.Ct. at 1739, 82 USPQ2d at 1395 (citing Graham v. John Deere Co., 383 U.S. 1, 12 (1966) (emphasis added)), and reaffirmed principles based on its precedent that "[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." Id. The Court explained:

> When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. If a person of ordinary skill can implement a predictable variation, §103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.

Id. at 1740, 82 USPQ2d at 1396. The operative question in this "functional approach" is thus "whether the improvement is more than the predictable use of prior art elements according to their established functions." *Id.*

The Court explained, "[o]ften, it will be necessary for a court to look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *Id.* at 1740-41, 82 USPQ2d at 1396. The Court noted that "[t]o facilitate review, this analysis should be made explicit." *Id.*, citing In re Kahn, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006) ("[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness"). However, "the analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *Id.*

a. Ball et al., the primary reference, does not teach, nor motivate, nor suggest, nor does it provide any articulable reasoning with any rational underpinning to support the combination which the Examiner asserts as obvious.

The Ball et al. reference is equivalent to U.S. Patent No. 6,008,340. Applicants refer the Board to the following excerpt which captures the entire teaching of Ball *et al.* with respect to fusion proteins of Phl pl epitopes and expressible polypeptides.

> A fourth aspect of the invention is a recombinant or synthetic protein or polypeptide displaying the antigenicity of a Phl p 1 epitope, in particular comprising as an essential part a Phl p I epitope of at least one of the sequences set out in SEQ ID NOS: 5, 7 and 9-28. The protein or polypeptides may be fused to an additional polypeptide, such as beta galactosidase, GST or lambda cII protein or **any other polypeptide that**

can be expressed as a **fusion protein** in prokaryotic or eukaryotic cells.

U.S. 6,008,340 Col. 2, In 64 -67; Col. 3, In 1-6. As admitted by the Examiner, Ball et al. do not teach fusion proteins consisting of two or more timothy grass pollen allergens. But under the new KSR regime, the Examiner need not find a teaching, suggestion or motivation; the Examiner may find an articulable reasoning with any rational underpinning to support the combination.

Applicants believe that the use of Ball et al. which teaches the fusion of galactosidase with Phl p 1 does not rationally support the combination which the Examiner asserts to be obvious. Indeed the fusion of proteins of interest with coordinate proteins which can enhance the expressibility of the fused protein complex is a well known and often utilized technique to boost prokaryotic gene expression. In this case, the protein of interest is fused with galactosidase and inserted downstream the operon promoter sequences in the galactose operon. Then subsequent administration of isopropyl β-thiogalactoside in liquid culture is used to induce the over-expression of galactosidase and the protein of interest fused with it.

The technique of amplifying expression of desirable proteins by fusing them with expressible proteins is old and well known. The Board is referred to the following teaching from the Ball *et al.* patent.

> The Phl p I epitope encoded by clone 98 was expressed as a beta - galactosidase fusion protein in liquid culture **(Huynh et al., 1985)** and was affinity purified using an anti-beta-galactosidase affinity column. (Promega, Maddison, USA) as described (Vrtala et al., 1993a).

U.S. 6,008,340 Col. 5, In 59 -65. The Huynh et al., (1985) reference cited in *Ball et al.* at least stands for the teaching that the fusion of proteins with expressible proteins is not new and has been the practice since 1985 and Ball et al. taught no more than the fusion of Phl p1 epitopes with expressible proteins merely to amplify the expression of, and to aid in isolation of proteins of therapeutic interest.

The rational inquiry under KSR is not whether Ball et al. taught the fusion of timothy grass pollen allergen with an expression booster protein; but whether Ball et al. can rationally stand for any teaching that fused timothy grass pollen allergens can and do have immunotherapeutic benefits. Prior to the current invention, no one has taught nor suggested that the fusion of hybrid allergens can produce immunotherapeutic agents more desirable than the respective component allergens. That such is the case was indeed a surprise to the inventors who are leading researchers in this area, and is in fact a surprise to anyone who is abreast with the developments in this art area. The Rule 132 declaration submitted prior affirmed the inventor's surprise that fusion proteins of naturally occurring allergens can be used as immunotherapeutic agents and exhibit increased immunogenicity. See Rule 132 declaration dated September 28, 2004. That Graham factor of surprise coupled with long felt obvious need to cross-sensitize patients against a broad spectrum of allergens with administration of the least amount of immunotherapeutic agents capable of inducing anaphylactic side effects, all tend to negate a rational underpinning for the use of Ball et al. against the current invention.

b. Vrtala *et al.*, the secondary reference, does not teach, nor motivate, nor suggest, nor does it provide any articulable reasoning with any rational underpinning to support the combination which the Examiner asserts as obvious.

The Examiner asserts that Vrtala et al. teach that "fusion polypeptides do not significantly affect the allergens IgE-binding capacity (page 782, col.1)." June 20, 2007, Office Action p 16. The Examiner further asserts that Vrtala et al., "teach protein having at least two timothy grass pollen allergens." Id. at 16. Applicants respectfully traverse.

Either the Examiner did not advert her mind closely to the teachings of Vrtala et al., or she is erroneously applying impermissible hindsight to reconstruct Vrtala et al. in order to tease out an articulable rationale to support the combination which the Examiner asserts as obvious.

Applicants draw the Boards attention to the abstract where Vrtala et al. teaches:

> Methods: The three recombinant grass pollen allergens were expressed at high levels in E. Coli as recombinant **nonfusion proteins**, purified by conventional protein chemical methods and tested for their IgE-binding capacity ...

Vrtala et al. p. 781, Abstract. When the Examiner asserted that Vrtala *et a*l. teach that "fusion polypeptides do not significantly affect the allergens IgE-binding capacity (page 782,col. 1)", the Examiner apparently did not advert her mind to the teaching on page 781, col. 2 that "in a plaque lift technique **β-galactosidase fusion proteins** of the above mentioned grass pollen allergens were successfully used to diagnose grass pollen allergy ..." Nor did the Examiner advert her mind to the teaching on page 782, col. 1 that "previous assays were done with recombinant **β-galactosidase fusion allergens**, which in addition to the mature protein contained the leader peptides and a large portion of β-galactosidase."

Vrtala et al. on page 782, col. 1, were apparently concerned that "although the [B-galactosidase] fused polypeptide did not significantly affect the **allergens'** IgE-binding capacity, the purification protocol had delivered rather small amounts of the recombinant **allergens."** (Emphasis added) Clearly, Vrtala et al. was speaking in terms of B-galactosidase fused allergens much like Ball et al.

The rest of Vrtala et al. on page 782 columns 1 and 2 went further to teach how to construct expression plasmids for each of Phl p1, Phl p2, and Phl p 5; expressing them in cell culture using isopropyl B-thiogalactoside as an inducer and the purification of the recombinant allergens. Throughout the disclosure, Vrtala et al. spoke in terms of a plurality of allergens and Applicants cannot find where the Examiner got the support that Vrtala et al. taught the fusion of timothy grass pollen allergens for use as immunotherapeutic agents.

That fused allergens can have immunotherapeutic benefits was a surprise as earlier mentioned. The Rule 132 declaration submitted prior affirmed the inventor's surprise that fusion proteins of naturally occurring allergens can be used as immunotherapeutic agents and exhibit increased immunogenicity. See Rule 132 declaration dated September 28, 2004. That Graham factor of surprise coupled with long felt obvious need to cross-sensitize patients against a broad

spectrum of allergens with administration of the least amount of immunotherapeutic agents capable of inducing anaphylactic side effects, all tend to negate a rational underpinning for the use of Ball et al. against the current invention.

Because the Examiner has not articulated a rational basis for the combination which she asserts as obvious, Applicants believe that this basis for rejection should be dropped.

CONCLUSION

In view of the forgoing discussion, it is respectfully submitted that the Examiner's rejections of claims 42 to 43, 45 - 47 (Groups 1 to 4) are improper and should be reversed by the Board.

Respectfully submitted,

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IX. Claims Appendix

1 – 6. (Canceled).

7. (Withdrawn) A polynucleotide encoding the hybrid polypeptide of claim 1.

8. (Cancelled).

9. (Withdrawn) A cell transfected or transformed with the polynucleotide of claim 7.

10 - 21. (Cancelled).

22. (Withdrawn) A method for treating an allergic disorder comprising administering the pharmaceutical composition of claim 20 to a patient in need thereof.

23. (Withdrawn) A method for inducing tolerance to a given allergen, comprising administering the pharmaceutical composition of claim 20 to a patient in need thereof.

24. (Withdrawn) A method for providing immunity to a given allergen, comprising administering the pharmaceutical composition of claim 20 to a patient in need thereof.

25. (Withdrawn) A method for detecting antibodies against a given allergenic protein in a sample, comprising conducting *in vitro* antibody tests employing the hybrid polypeptide of any one of claims 1 to 6 or conducting *in vitro* or *in vivo* cellular-based tests employing the hybrid polypeptide of any one of claims 1 to 6.

26 – 35. (Cancelled).

36 (Withdrawn) A method of identifying plant hybrid allergens for treatment of IgE-mediated

hypersensitivity to the respective wild-type allergens comprising the steps of:

(a) providing a fusion allergen of naturally occurring plant allergens;

(b) challenging an immunological model with said fusion allergen;

(c) selecting as candidate immunotherapeutic agents, those fusion allergens which induce IgE-blocking antibodies and have reduced allergenic activity compared with the respective wild-type allergens.

37. (Withdrawn) The method of claim 36, wherein the hybrid allergen is a fusion protein of two or more wild-type allergens.

38. (Withdrawn) The method of claim 36, wherein the hybrid allergen is a fusion protein of fragments of two or more wild-type allergens.

39. (Withdrawn) The method of claim 36, wherein the hybrid allergen is a fusion protein of fragments of two or more wild-type allergens, and wherein each fragment contains at least eight consecutive amino acids of the wild-type allergen.

40. (Withdrawn) The method of claim 37, wherein the hybrid allergen is a fusion protein of one or more modifications of at least one of the two or more wild-type allergens.

41. (Withdrawn) The method of claim 36, wherein the hybrid allergen is prepared by chemical synthesis.

42. (Previously presented) A method of preparing fusion polypeptides consisting of timothy grass pollen allergens for use as immunotherapeutic agents comprising:

(a) providing a polynucleotide sequence encoding the fusion polypeptide;

(b) introducing said polynucleotide sequence into a host cell;

(c) culturing the host cell obtained in b) under conditions such that the fusion polypeptide is expressed; and

(d) recovering the expressed fusion polypeptide from the cultured host cell;

(e) testing the fusion polypeptide as candidate immunotherapeutic agents by administering

said polypeptide to a test animal and selecting as immunotherapeutic agents those fusion polypeptides that induce IgE-blocking antibodies and induce stronger immune responses compared with the individual components or fragments thereof.

43. (Previously presented) The method of claim 42, wherein the polynucleotide *sequence* encoding the timothy grass pollen polypeptide is obtained using PCR technology.

44. (Withdrawn) A method of treating IgE-mediated hypersensitivity to plant allergens comprising administering to a patient in need of such treatment, a pharmaceutical composition comprising one or more hybrid plant fusion allergens as immunotherapeutic agents, wherein said agents have been identified by a method comprising the steps of:

(a) providing fusion allergens of naturally occurring plant allergens;

(b) challenging an immunological model with said fusion allergen;

(c) selecting as candidate immunotherapeutic agents, those fusion allergens which induce IgE-blocking antibodies and have reduced allergenic activity compared with the respective wild-type allergens.

45. (Previously presented) A pharmaceutical composition comprising one or more fusion allergens of timothy grass pollen allergens as immunotherapeutic agents, wherein said agents consists of fusion allergens of timothy grass pollen allergens which have been identified by a method comprising the steps of:

(a) providing fusion allergens of naturally occurring timothy grass pollen allergens;

(b) challenging an immunological model with said fusion allergens;

(c) selecting as candidate immunotherapeutic agents, those fusion allergens which induce IgE-blocking antibodies and have reduced allergenic activity compared with the respective allergens which comprise the fusion allergen.

46. (Previously presented) A hybrid allergen for treatment of IgE-mediated hypersensitivity, wherein said hybrid allergen is a fusion protein consisting of two or more timothy grass pollen allergens.

47. (Previously presented) The hybrid allergen of claim 46, wherein said hybrid allergen is a fusion protein of two or more proteins selected from the group consisting of timothy grass pollen allergens rPhl p 1, rPhl p 2, rPhl p 5, and rPhl p 6.

48 - 51. (Canceled).

X. Evidence Appendix

Tab 1 Ball et al. (WO 95/34578); equivalent to U.S. 6,008,340 and was entered in the record by the Examiner on January 04, 2005.

Tab 2. Vrtala et al. 1996. J. Allergy Clin. Immun., Vol. 97(3): 781 - 787), entered into record as part of Information Disclosure Statement filed June 24, 2002.

Tab 3. Declaration under Rule 132 entered into the record as part of Response and Amendment to Office Action filed August 07, 2006.

XI. Related Proceedings Appendix

None.

FIK 4

Immunologic characterization of purified recombinant timothy grass pollen *(Phleum pratense)* allergens (Phl p 1, Phl p 2, Phl p 5)

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Background: *Grass pollen allergens belong to the potent elicitors of type I allergy. Approximately 40% of allergic individuals display IgE reactivity with grass pollen allergens. In previous studies we have reported the complementary DNA cloning and expression in* Escherichia coli *of three of the most relevant timothy grass pollen allergens: Phl p 1, Phl p 2, and Phl p 5.*
Objective: *To achieve high level expression of immunologically active timothy grass pollen allergens in* E. coli, *the cDNAs were inserted into expression plasmids.*
Methods: *The three recombinant grass pollen allergens were expressed at high levels in* E. coli *as recombinant nonfusion proteins, purified by conventional protein chemical methods and tested for their IgE-binding capacity by immunoblot and ELISA, as well as in histamine release assays.*
Results: *Milligram amounts of pure recombinant allergens were obtained from cultured* E. coli. *IgE binding to purified recombinant Phl p 1, Phl p 2, and Phl p 5 could be demonstrated by immunoblot and ELISA. With ELISAs the percentage of grass pollen–specific IgE directed against the individual recombinant allergens could be estimated. In addition, the purified recombinant timothy grass pollen allergens induced dose-dependent and specific histamine release from patients' blood basophils.*
Conclusion: *Purified recombinant timothy grass pollen allergens represent useful tools for diagnosis and therapy of grass pollen allergy. (J ALLERGY CLIN IMMUNOL 1996;97:781-7.)*

Key words: Recombinant timothy grass pollen allergens, Phl p 1, Phl p 2, Phl p 5, high level expression in E. coli, *histamine release*

Type I allergic reactions caused by grass pollen allergens occur worldwide. Groups of grass pollen allergens with extensive immunologic cross-reactivities have been described in various grass species.[1-3] We have previously isolated complementary DNAs coding for three major timothy grass pollen allergens representing group I (Phl p 1),[4] group II (Phl p 2),[5] and group V (Phl p 5).[6] Phl p 1 is a target for IgE antibodies in more than 95% of patients with grass pollen allergy. Phl p 5, though bound by fewer patients (80% of patients with grass pollen allergy) is of particular importance because of its extremely high IgE-binding capacity; and Phl p 2 represents a low molecular weight allergen (10 kd) for 60% of the patients, and although similar in sequence to Phl p 1, shows no relevant immunologic similarity to Phl p 1. In a plaque lift technique β-galactosidase fusion proteins of the above-mentioned grass pollen allergens were successfully used to diagnose grass pollen allergy in a representative number of individuals,[7] and it was further demonstrated that recombinant Phl p 1, Phl p 2, and Phl p 5, as well as timothy grass profilin (Phl p 11),[8] can be used to precisely determine the sensitization patterns of these patients with

Abbreviations used
BSA: Bovine serum albumin
DFP: Diisopropyl fluorophosphate
PBS: Phosphate-buffered saline

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allergy (allergogram).[9] In addition, it was demonstrated that recombinant timothy grass pollen allergens bound a high proportion of grass pollen–specific IgE.[9] However, the previous assays were done with recombinant β-galactosidase fusion allergens, which in addition to the mature protein contained the leader peptides and a large portion of β-galactosidase. Although the fused polypeptide did not significantly affect the allergens' IgE-binding capacity, the purification protocols had delivered rather small amounts of the recombinant allergens. In this study we have modified the allergen-encoding cDNAs so that milligram amounts of functional nonfusion allergens were expressed in *Escherichia coli*.[10] The recombinant grass pollen allergens were tested for antibody binding and specific histamine release and were found to behave similarly to their natural counterparts. A considerable improvement of in vitro and in vivo diagnosis of grass pollen allergy is expected if recombinant allergens can be used for a routine determination of the sensitization patterns in patients with grass pollen allergy. Allergen-specific diagnosis may consecutively improve specific immunotherapy.

METHODS

Characterization of patients with timothy grass pollen allergy

Patients allergic to grass pollen in this study were evaluated by case history, RAST (Pharmacia, Uppsala, Sweden) and skin prick test. Sera from allergic individuals had been tested with natural and recombinant timothy grass pollen allergens and were selected from 100 tested sera to reflect a representative binding pattern.[7, 9]

Construction of the expression plasmids for Phl p 1, Phl p 2, and Phl p 5

The cDNA clones of Phl p 1, Phl p 2, and Phl p 5[4-6] were transcribed by polymerase chain reaction to DNA fragments coding for the mature allergens. The signal peptides were replaced by a methionine by placing an *Nde* I (Phl p 1, Phl p 2) or an *Nco* I (Phl p 5) restriction site, both of which contain the ATG codon, in front of the coding region of the mature protein. An *Eco R* I site was introduced immediately after the stop codon. The genes were inserted as *Nde* I/*Eco R* I (Phl p 1, Phl p 2) or *Nco* I/*Eco R* I (Phl p 5) fragments into pMW 172[11] (Phl p 1, Phl p 2) a derivative of pRK 172[12] or pMW 175 (Phl p 5), a derivative of pMW 172 where the *Nde* I site is replaced by *Nco* I. The plasmids were transfected into *E. coli* BL 21 (DE3) with the calcium chloride method.[13] BL 21 is derived from *E. coli* strain B.[14]

Expression of recombinant allergens in *E. coli*

Recombinant Phl p 1, rPhl p 2, and rPhl p 5 were expressed in *E. coli* BL21 (DE3) in liquid culture on induction with isopropyl β-thiogalactoside (0.2 to 0.4 mmol/L) when cells were grown to an optical density of 0.8 to 1.2. Yields of 28 mg (Phl p 1), 3 mg (Phl p 2), and 14 mg (Phl p 5) per gram wet weight of cells were obtained in 150 ml cultures. The amount of proteins was estimated from band intensities of Coomassie blue–stained sodium dodecylsulfate polyacrylamide electrophoresis gels (Coomassie blue; Sigma Chemical Co., St. Louis, Mo.).

Purification of recombinant allergens

Phl p 1. Phl p 1 was expressed in inclusion bodies, which were isolated after cells were thawed and suspended in 10 mmol/L Tris (pH 8), 2 mmol/L β-mercaptoethanol, 0.1% vol/vol Triton X-100, 0.5 mmol/L ethylenediaminetetraacetic acid (5 ml buffer/gm cells). Cell lysis was performed by adding lysozyme (20 μg/gm cells) for 30 minutes at room temperature. Ca^{2+} was then added to 3 mmol/L final concentration, and the mixture was then digested with deoxyribonuclease I (0.1 mg/gm) for an additional 30 minutes at room temperature. The volume was then doubled by addition of 20 mmol/L Tris pH 8, 1% wt/vol desoxycholate, 2 mmol/L ethyleneglycol-bis-(β-aminoethylether)-N,N,N′,N′,-tetraacetic acid, and 0.2 mol/L NaCl (buffer P). The lysate was then centrifuged (5 minutes, 8000 *g*) and the pellet washed four times with fourfold-diluted buffer P containing 0.05 mmol/L diisopropyl fluorophosphate (DFP) by centrifugation. The resulting pellet was finally washed with 10 mmol/L Tris pH 8, 3% vol/vol isopropanol to remove residual detergents.

Inclusion bodies were then solubilized with 8 mol/L urea, 10 mmol/L Tris pH 8, 1 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L β-mercaptoethanol, 0.05 mmol/L DFP (5 ml/gm cells) and dialyzed against the same buffer without urea. The dialyzed sample was then centrifuged for 10 minutes at 8000 *g* to remove precipitated material (containing approximately 20% to 30% of the recombinant Phl p 1), and the supernatant was then adjusted to 150 mmol/L NaCl and dialyzed against 100 mmol/L NaCl, 20 mmol/L NaH_2PO_4, 0.05 mmol/L DFP without adjustment of pH. After dialysis, the supernatant was extracted with 0.5% vol/vol of chloroform, and the aqueous phase was precipitated with ammonium sulfate 60% wt/vol. A final purification step with a diethylaminoethyl cellulose column (M. Susani, et al. Unpublished data) yielded a soluble protein that was more than 95% pure.

Phl p 2. Phl p 2 was expressed as soluble protein. Cells were lysed as described above and centrifuged for 15 minutes at 14,000 *g;* the supernatant was brought to 0.05 mmol/L DFP. The supernatant was then dialyzed against 50 mmol/L phosphate buffer, pH 4.5, overnight. After centrifugation at 14,000 *g* for 15 minutes, the supernatant was brought to pH 5.5 with NaOH, and ammonium sulfate was added to 65% wt/vol. After another centrifugation (14,000 *g*, 15 minutes), the supernatant was applied to a phenyl-Sepharose column (Pharmacia), and the protein was eluted with 1 mol/L ammonium sulfate. The protein was then dialyzed against 25 mmol/L imidazole, pH 7.4, and rechromatographed on a diethylami-

noethyl cellulose–Sepharose column to yield a protein more than 98% pure.

Phl p 5. E. coli cells were lysed as described above. After centrifugation (15 minutes, 14,000 *g*), the supernatant was adjusted to pH 6.0 with HCl. Material that was precipitated by addition of ammonium sulfate between 30% and 50% wt/vol was used for further purification. The precipitate was dissolved in 10 mmol/L phosphate, 0.1 mmol/L dithiothreitol, and 0.1% Triton X-100, pH 5.0, then dialyzed against the same buffer without Triton X-100 and applied to SP-Sepharose. Fractions containing more than 90% pure Phl p 5 were concentrated by ammonium sulfate precipitation, dissolved in 100 mmol/L phosphate, pH 5.3, brought to 0.7 mol/L ammonium sulfate and chromatographed on a PS-Sepharose column to yield a protein of more than 99% purity.

IgE binding to natural timothy grass pollen extracts and purified recombinant timothy grass pollen allergens (Phl p 1, Phl p 2, and Phl p 5)

IgE binding to timothy grass pollen allergens was measured by immunoblotting and ELISA. Nitrocellulose strips, containing total timothy grass pollen proteins, purified rPhl p 1, purified rPhl p 2, or purified rPhl p 5 were incubated with sera from 13 different representative patients with timothy grass pollen allergy. Patients' sera were diluted 1:10 in buffer G (50 mmol/L sodium phosphate, pH 7.5, 0.5% Tween-20, 0.5% bovine serum albumin [BSA], 0.05% NaN_3), and bound IgE was detected with iodine 125–labeled anti-human IgE (Pharmacia) as described by Valenta et al.[15] For semiquantitative estimation of specific IgE against total timothy grass pollen proteins and purified recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 2, and rPhl p 5) an ELISA was used. Amounts of allergens and serum dilutions were used so that antigens were in excess of IgE and IgG antibodies to allow the determination of the relative percentage of grass pollen–specific antibodies directed against the recombinant allergens. The optimal amounts of allergens and serum dilutions were determined in pilot experiments.

ELISA plates were coated with 50 μg/ml total timothy grass pollen proteins or purified, recombinant allergens (2 μg/ml). The plates were washed two times with phosphate-buffered saline (PBS), 0.05% Tween-20, blocked for 2.5 hours with PBS, 1% BSA, 0.05% Tween-20 at room temperature and incubated overnight at 4° C with sera from patients with grass pollen allergy. Patients' sera were diluted 1:20 in PBS, 0.5% BSA, 0.05% Tween-20 for the detection of specific IgE and 1:100 for detection of IgG_1, IgG_2, IgG_3, and IgG_4. The plates were washed five times with PBS, 0.05% Tween-20, and bound immunoglobulins were detected with monoclonal mouse anti-human Ig antibodies (PharMingen, San Diego, Calif.), diluted 1:1000 in PBS, 0.5% BSA, 0.05% Tween-20 overnight at 4° C. The plates were washed five times with PBS, 0.05% Tween-20 and incubated for 30 minutes at 37° C and 30 minutes at 4° C with a horseradish-peroxidase–coupled sheep anti-mouse antiserum (Amersham, Buckinghamshire, U.K), diluted 1:2000 in PBS, 0.5% BSA, 0.05% Tween-20. Plates were again washed five times with PBS, 0.05% Tween-20, and ABTS (60 mmol/L citric acid, 77 mmol/L $Na_2HPO_4.2H_2O$, 1.7 mmol/L ABTS [Sigma, St. Louis, Mo.], 3 mmol/L H_2O_2) was added. Plates were incubated in the dark for 30 minutes at room temperature, and the color reaction was stopped by addition of 100 μl/well 0.32% NaF. Extinctions (optical density 450 to 405 nm) were determined with an ELISA reader (Dynatech, Denkendorf, Germany).

In vitro histamine release assays with purified recombinant allergens

Heparinized blood samples were obtained from patients with grass pollen allergy after informed consent had been given. Granulocytes were prepared by dextran sedimentation and washed and incubated with various doses of recombinant allergens, anti-human IgE, or buffer as previously described.[16] Liberated histamine was measured in the cell-free supernatants by radioimmunoassay (Immunotech, Marseille, France).

RESULTS

Expression in *E. coli* and purification of recombinant timothy grass pollen allergens

In a previous study recombinant timothy grass pollen allergens were expressed as β-galactosidase fusion proteins.[9] In an attempt to obtain recombinant nonfusion allergens, which are similar to the natural allergens, polymerase chain reaction primers were synthesized according to the Phl p 1, Phl p 2, and Phl p 5 cDNA sequences to allow the amplification of cDNAs without the hydrophobic leader sequences that are absent in the mature proteins. The cDNAs coding for the mature allergens were amplified from the allergen cDNAs and subcloned into plasmid pET. Recombinant nonfusion proteins were expressed in *E. coli* BL21 (DE3) on induction with IPTG and purified. Fig. 1 shows a Coomassie blue–stained protein gel containing total timothy grass pollen extract in lane P and purified recombinant timothy grass pollen allergens (lanes I, II, and V). Milligram amounts of more than 90% pure and water-soluble recombinant Phl p 1, Phl p 2, and Phl p 5 were obtained for subsequent immunologic testing.

IgE-binding capacity of recombinant timothy grass pollen allergens

Purified recombinant timothy grass pollen allergens were tested for their antibody-binding capacity in denaturing and nondenaturing assays. Nitrocellulose-blotted natural timothy grass pollen

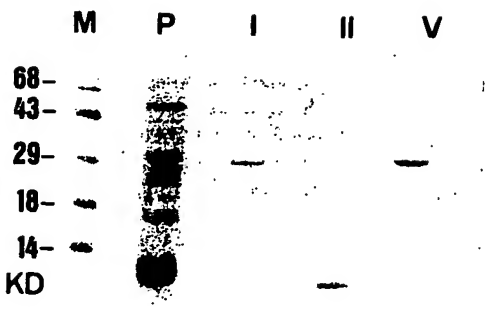

FIG. 1. Coomassie brilliant blue–stained polyacrylamide gel. *Lane M* represents a molecular weight marker; *lane P* shows a protein extract from timothy grass pollen; *lane I*, purified rPhl p 1; *lane II*, purified rPhl p 2; and *lane-V*, purified rPhl p 5.

allergens and purified recombinant timothy grass pollen allergens were compared for IgE binding by using sera from 13 patients with grass pollen allergy. The sera were selected to represent the typical IgE-binding pattern to timothy grass pollen allergens from 100 individuals with grass pollen allergy as determined by case history, RAST, and skin prick testing.[7, 9] With the exception of patient 6 (Fig. 2), all patients showed IgE reactivity with nitrocellulose-blotted natural timothy grass pollen allergens. IgE binding was observed in the 30 kd range with group I and group V allergens and at around 10 kd with group II allergens. Two patients (nos. 2 and 4) showed weak reactivity to group IV allergens at 54 kd. All patients, including patient 6 who had no detectable IgE against natural timothy grass pollen allergens, displayed IgE reactivity to recombinant Phl p 1. Twelve of 13 patients reacted with recombinant Phl p 5, and 10 patients had IgE against recombinant Phl p 2. Recombinant timothy grass pollen allergens thus allowed differentiation among IgE reactivity to group I, group II, and group V allergens, which cannot be distinguished when nitrocellulose-blotted natural grass pollen extracts are used.

The ELISA results (Fig. 3) showed that in most of the patients high levels of timothy-specific IgE were directed against Phl p 5 and Phl p 1. Phl p 2 bound rather low levels of specific IgE. The reactivity in the different IgG subclasses with a given allergen was not always associated with its IgE-binding capacity (Fig. 3).

Recombinant timothy grass pollen allergens induce specific and dose-dependent histamine release

To demonstrate specific histamine release capacity of recombinant timothy grass pollen allergens, basophils from patients with grass pollen allergy were incubated with recombinant Phl p 1, Phl p 2, or Phl p 5. Figs. 4 and 5 show that purified recombinant timothy grass pollen allergens induced specific and dose-dependent histamine release in patients with grass pollen allergy. To ensure that specific IgE antibodies were present in the patients' sera at the time when the histamine release assays were done, supernatants obtained during the enrichment of basophils were probed for IgE reactivity with natural and recombinant timothy grass pollen allergens (Figs. 2 and 3). Comparing the IgE reactivity of the patients displayed in the immunoblots in Fig. 2 with the histamine release capacity of recombinant allergens, we found that all patients with IgE antibodies specific for the recombinant allergen tested also had positive histamine release results. The optimal dose of recombinant timothy grass pollen allergens for inducing maximal histamine release was usually approximately 1 μg/ml. As in previous studies performed with recombinant fusion allergens and natural allergens, histamine release was observed at doses as low as 10 ng/ml allergen.[6] The recombinant allergens were also tested with basophils from patients without specific IgE antibodies and were found to induce no histamine release up to concentrations of 10 μg/ml recombinant allergen (data not shown). Negative controls performed without addition of recombinant allergens or recombinant allergens against which patients were not sensitized did not lead to histamine release, whereas by using a monoclonal anti-human IgE antibody (positive control), histamine release could be induced in all patients tested as previously described.[16]

DISCUSSION

A number of cDNAs coding for plant, mite, animal, and fungal allergens have been characterized.[17, 18] Many of these cDNAs were obtained by IgE immunoscreening of expression cDNA libraries,[18] and it was thus possible to test the bacterially expressed allergens for their IgE-binding capacity with sera from allergic patients. In previous studies the molecular cloning and expression of cDNAs coding for three major timothy grass pollen allergens (Phl p 1, Phl p 2, and Phl p 5) has been reported.[4-6] With β-galactosidase–fused recombinant timothy grass pollen allergens it was shown that a panel of only three to four recombinant timothy grass pollen allergens can be sufficient to diagnose grass pollen allergy.[7] In another study it has been demonstrated that a combination of these

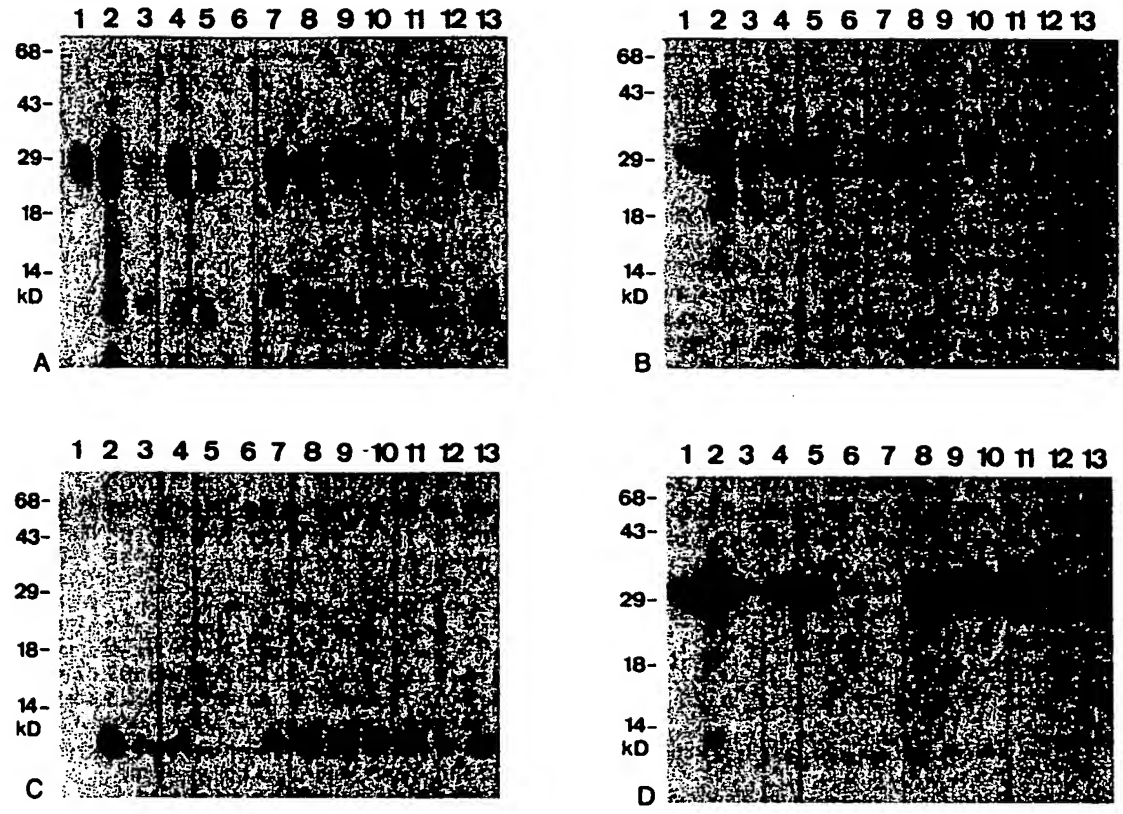

FIG. 2. IgE immunoblots with total timothy grass pollen proteins **(A)**, purified rPhl p 1 **(B)**, purified rPhl p 2 **(C)**, and purified rPhl p 5 **(D)**. Thirteen patients with timothy grass pollen allergy *(lanes 1 to 13)* were tested for IgE binding to nitrocellulose-blotted natural timothy grass pollen proteins **(A)** and purified recombinant timothy grass pollen allergens **(B, C, and D)**.

three recombinant timothy grass pollen allergens bound a high percentage of grass pollen–specific IgE.[9] Although β-galactosidase–fused timothy grass pollen allergens showed similar IgE-binding capacity compared with natural timothy grass pollen allergens, the expression of large amounts of rPhl p 1, rPhl p 2, and rPhl p 5 without leader peptides and fused protein was attempted. In this study the expression of functional rPhl p 1, rPhl p 2, and rPhl p 5 is reported. The recombinant allergens lack their hydrophobic leader peptides and other additionally fused polypeptides. In contrast to the natural allergens, an additional methionine is present at the N-terminus, and no carbohydrates or hydroxyprolines are present in the recombinant proteins because of the bacterial expression system used. As already demonstrated for the β-galactosidase–fused allergens, IgE binding to the native and denatured allergens was shown. The use of rPhl p 1, rPhl p 2, and rPhl p 5 allowed a precise determination of the patients' sensitization patterns. A major advantage of recombinant allergens is the possibility to discriminate patients' IgE reactivity to allergens of a similar molecular weight, such as Phl p 1 or Phl p 5. Patient 6, who showed no detectable IgE reactivity to nitrocellulose-blotted natural grass pollen extract, was found to react with recombinant Phl p 1. Although considerable amounts of natural grass pollen extracts had been used to coat the ELISA plates, a combination of the three recombinant timothy grass pollen allergens yielded, for the most part, a higher extinction than the natural extract. Recombinant allergens therefore offer the advantage of measuring allergen-specific antibody levels, and it appears that carbohydrate moieties or amino acid modifications, such as hydroxyprolines, which frequently occur in natural grass pollen allergens, are not of relevance for patients' IgE binding.

In addition to the antibody-binding capacity, the biologic activity of rPhl p 1, rPhl p 2, and rPhl p 5

#	1	2	3	4	5	6	7	8	9	10	11	12	13	
E	0.16	2.26	0.18	0.40	0.44	0.05	0.36	0.34	0.88	0.22	0.78	0.16	0.24	
I	0.10	0.52	0.11	0.10	0.29	0.05	0.29	0.13	0.47	0.13	0.16	0.09	0.10	IgE
II	0.08	0.19	0.09	0.05	0.05	0.04	0.12	0.10	0.13	0.06	0.12	0.05	0.07	
V	0.17	1.54	0.14	0.43	0.34	0.05	0.06	0.32	1.33	0.18	0.99	0.16	0.24	
E	0.15	0.60	0.50	0.28	0.20	0.09	0.16	0.73	0.20	0.13	0.47	0.26	0.25	
I	0.08	0.28	0.27	0.11	0.10	0.10	0.12	0.58	0.18	0.11	0.10	0.16	0.20	IgG_1
II	1.09	0.25	0.31	0.09	0.07	0.12	0.38	0.38	0.10	0.15	0.08	0.19	0.13	
V	0.10	0.45	0.48	0.30	0.09	0.10	0.10	0.81	0.65	0.16	0.14	0.25	0.19	
E	0.23	0.63	1.19	0.51	0.34	0.14	0.20	1.77	0.50	0.21	1.18	0.28	0.14	
I	0.23	0.50	0.64	1.13	0.61	0.94	0.64	0.55	1.04	0.24	1.32	1.16	0.36	IgG_2
II	1.01	0.19	0.48	0.47	0.29	0.34	0.24	0.58	0.71	0.44	0.54	0.54	0.46	
V	0.14	0.20	0.66	0.25	0.21	0.12	0.14	1.25	0.83	0.18	0.37	0.47	0.13	
E	0.11	0.28	0.12	0.08	0.13	0.15	0.12	0.35	0.13	0.08	2.04	0.07	0.09	
I	0.06	0.05	0.05	0.05	0.06	0.13	0.09	0.08	0.09	0.05	0.08	0.05	0.07	IgG_3
II	0.09	0.05	0.06	0.06	0.11	0.06	0.04	0.05	0.70	0.37	0.11	0.05	0.05	
V	0.26	0.07	0.05	0.05	0.06	0.11	0.05	0.09	0.08	0.28	0.07	0.58	0.05	
E	0.27	1.95	>2.5	2.15	0.22	0.06	0.28	>2.5	0.29	0.75	0.42	0.13	0.14	
I	0.08	0.40	2.31	0.36	0.21	0.06	0.18	2.05	0.16	0.35	0.27	0.08	0.10	IgG_4
II	0.20	0.50	>2.5	0.19	0.07	0.07	0.12	>2.5	0.17	0.27	0.14	0.06	0.07	
V	0.14	0.58	>2.5	1.10	0.20	0.05	0.06	>2.5	0.33	0.54	0.48	0.10	0.15	

FIG. 3. IgE, IgG_1-IgG_4 reactivity to natural *(E)* and recombinant timothy grass pollen allergens (*I*, Phl p 1; *II*, Phl p 2; *V*, Phl p 5) estimated by ELISA in 13 patients with grass pollen allergy. Extinctions over baselines determined with a group of nonallergic individuals are displayed.

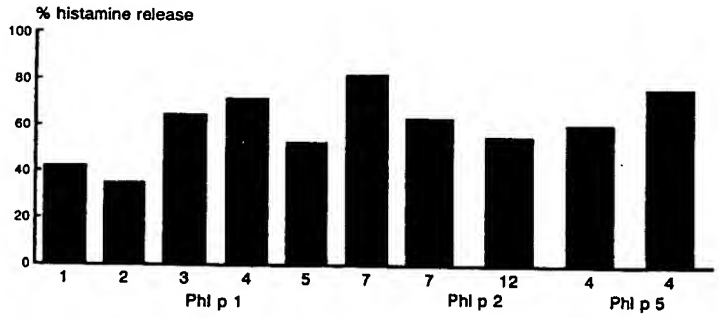

FIG. 4. Induction of histamine release from basophils of patients with grass pollen allergy with purified recombinant Phl p 1, Phl p 2, and Phl p 5. Basophils from six patients allergic to Phl p 1 were incubated with recombinant Phl p 1; basophils from two donors were incubated with recombinant Phl p 2 and basophils from one patient allergic to Phl p 5 (two experiments with the same patient). The percentage of histamine release at the optimal allergen concentration (1 μg/ml) is displayed. Patient numbers are the same as in Fig. 2 to allow a comparison of histamine-releasing capacity and IgE-binding capacity of recombinant timothy grass pollen allergens.

was demonstrated in basophil degranulation assays. The basophil degranulation assay[19] has been shown to closely reflect the cascade of the allergic effector reaction, and in the case of recombinant birch pollen allergens, has already served as a valuable tool for testing recombinant allergens in a close to in vivo system.[15, 19] In this study purified recombinant timothy grass pollen allergens induced dose-dependent and specific histamine release from basophils of sensitized patients. Even at concentrations up to 10 μg/ml, no cell toxic effects were observed.

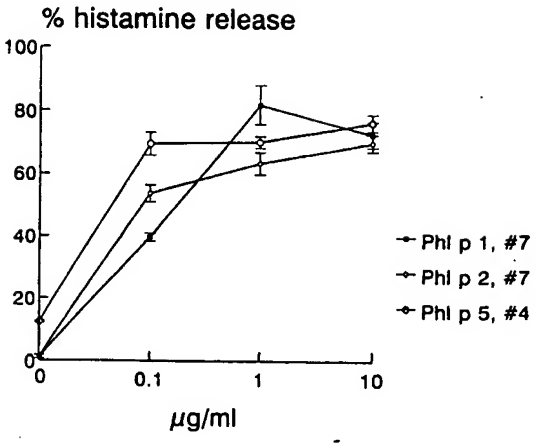

FIG. 5. Dose dependency of histamine release induced with basophils from representative patients allergic to grass pollen by use of recombinant Phl p 1, Phl p 2, and Phl p 5. Basophils from three representative patients with grass pollen allergy were incubated with different concentrations of recombinant timothy grass pollen allergens, and histamine release was determined in the cell-free supernatants by radioimmunoassay. Histamine release induced from basophils of patient 7 with rPhl p 1 and rPhl p 2 and the dose dependency of a histamine release obtained with recombinant Phl p 5 in patient 4 are shown. Patient numbers are the same as in Fig. 2.

In conclusion we believe that the expression of large amounts of recombinant timothy grass pollen allergens will substantially add to current forms of allergy diagnosis by allowing determination of the patients' sensitization patterns (allergogram). The recombinant nonfusion allergens can be produced in gram amounts with consistent batch-to-batch quality and can be used for current in vitro allergy tests, as well as for skin prick testing. On the basis of the determination of the patients' individual sensitization patterns and specific antibody levels, immunotherapy with selected recombinant allergens is a realistic prospect for the near future.[20]

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Linhart, *et al.*

Appl. No. 10/026,914
Filed: December 27, 2001

For: Allergy Vaccines Containing Hybrid Polypeptides

Art Unit: 1645
Examiner: Jana A. Hines
Atty. Docket: 966927.00006 (0273-0006)

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Professor Rudolf Valenta, am an associate professor at the Division of Immunopathology (Department of Pathophysiology, University of Vienna, Austria), and I do hereby declare and state that:

1. I studied medicine at the University of Vienna and graduated with an MD degree in 1987.

2. Since 1988 I have been working at the Department of Pathophysiology (formerly: Department of General and Experimental Pathology) at the University of Vienna, Austria.

3. I extended my scientific experience during trainings in molecular biological techniques in the labs of Markus Susani (Institute of Molecular Biology, Salzburg, Austria) and Michael Breitenbach (Department of Genetics and Microbiology, University of Vienna) in 1988.

4. In 1992, I was awarded the qualification of a University lecturer for general and experimental pathology and became Head of the Molecular Immunopathology group at the Division of Immunopathology.

5. After a training in cellular mouse immunology in the lab of Alec Sehon (Department of Immunology, University of Manitoba, Winnipeg, Canada) and a one year's specialist training in Internal medicine and design of clinical studies at the Department of Hematology and the Department of Clinical Pharmacology of the University of Vienna in the years 1994 and 1995, I was granted a specialist degree for Pathophysiology in 1996.

6. In 1997, I completed my specialist training in immunology and became associate professor for Pathophysiology.

7. I have been Head of the Division of Immunopathology since December 2001 and have since headed the special research program: Molecular and immunological strategies for prevention, diagnosis and treatment of Type I allergies.

8. I have contributed to more than 200 peer-reviewed scientific papers, reviews, and book Chapters.

9. My work on the characterization of allergens and their use for new concepts of allergy treatment was granted numerous scientific awards, among others: the Sandoz Austria Award for Biology in 1994; the International Award of the Pharmacia Allergy Research Foundation in 1996; the START Award of the Austrian Science Fund in 1998; and the Sarstedt Science Award in 2000.

10. I have read and understood the Examiner's basis for rejection of the claims of the above-captioned Application, in the June 15, 2006, Office Actions particularly the 35 U.S.C. § 103(a) rejection over Ball et al., (WO 95/34578) in view of Vrtala et al., (1996: J. Allerg Clin. Immun. Vol 97(3): 781-787).

11. I have read and understood the disclosure and teachings of the Ball et al. reference (Same as US 6,008,340) particularly in reference to fusion polypeptides of Phl p1 epitopes and one other additional polypeptide.

12. Essentially Ball et al. teaches the use of "fusion polypeptides of Phl p1 epitopes and one other polypeptide," wherein that other polypeptide is used to drive the expression process and purification process. (See US 6,008,340 Col. 3, lines 1-6; Col. 5, lines 59 -62; and Col. 8, lines 15-31).

13. Ball et al. failed to teach or suggest that the fusion polypeptide of Phl p1 epitopes and another polypeptide that can be expressed as a fusion protein in prokaryotic or eukaryotic cells can be used as an immunotherapeutic agent.

14. In fact, Ball et al. failed to teach or suggest the fusion polypeptide of Phl p1 epitopes with a second polypeptide, which is itself an allergen.

15. In fact, Ball et al. failed to teach or suggest the fusion polypeptide of Phl p1 epitopes to a second, third, fourth etch polypeptide, all of which are allergens.

16. In contrast, the present invention teaches that one or more recombinant produced timothy grass pollen allergens can be fused and said fusion protein can be used as immunotherapeutic agent and moreover that the immunogenicity of each of the components of the fusion protein are increased through the fusion described in the present invention.

17. In fact, the inventors are surprised that fusion proteins of naturally occurring allergens can be used as immunotherapeutic agents and exhibit increased immunogenicity.

18. This surprising result is the subject of much speculation as to whether the fusion of allergens in some way destroys certain epitopes in the fusion allergen compared to the native unfused allergens.

19. To validate this surprising discovery, we tested whether immunization with the fusion allergens induces IgG antibodies (IgE-blocking antibodies) that recognize the individual allergen components.

20. As demonstrated in Figure 5 of the instant specification, the average IgG1 responses induced by the hybrid molecules to each of the individual allergens (rPhl p1, rPhl p2, rPhl p5, rPhl p6) were higher than those obtained by immunization with the single allergen components.

21. To further validate this surprising discovery, we tested whether mouse antibodies induced with the hybrid molecules can block the binding of grass pollen allergic patient's IgE antibodies to purified grass pollen allergens.

22. As shown in Tables 3A and 3B of the instant specification, IgG antibodies induced with the rP2-P6 and the rP6-P2 fusion proteins caused a 48%-54% inhibition of IgE binding to Phl p2 and a 54% to 67% inhibition of IgE binding to Phl p6 (Table 3A). By contrast, the inhibition of IgE reactivity yielded by preincubation with antibodies induced with rPhl p2 and rPhl p6 alone was very low (0-15%). (Table 3A).

All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents ensuing thereon.

Respectfully submitted,

Date: 27. JULI, 2006

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